

REPORT DOCUMENTATION PAGE			Form Approved OMB NO. 0704-0188	
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1. AGENCY USE ONLY (Leave blank)	2. REPORT DATE October 14, 1996	3. REPORT TYPE AND DATES COVERED Final 1 Sep 92 - 31 Aug 96		
4. TITLE AND SUBTITLE Stereochemical Dependence of Vesicle Bilayer Properties		5. FUNDING NUMBERS DAAL03-92-G-0359		
6. AUTHOR(S) David A. Jaeger				
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9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Research Office P.O. Box 12211 Research Triangle Park, NC 27709-2211		10. SPONSORING / MONITORING AGENCY REPORT NUMBER ARO 30558.2-CH-EPS		
11. SUPPLEMENTARY NOTES The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision, unless so designated by other documentation.				
12a. DISTRIBUTION / AVAILABILITY STATEMENT Approved for public release; distribution unlimited.		12 b. DISTRIBUTION CODE 19961125 123		
13. ABSTRACT (Maximum 200 words) The project involved several studies of surfactants and their derived vesicles. The primary objective of the project was an elucidation of factors involved in the stabilization and destabilization of vesicles. Work was performed on three studies. The first involved the dependence of the properties of vesicles on the stereochemistry of their constituent surfactants. The second and third studies involved the synthesis of double-chain, double-head group surfactants and chain-substituted phosphocholines, respectively, and characterization of their derived vesicles. The primary methods used to characterize the vesicles were dynamic laser light scattering, to give sizes, and differential scanning calorimetry, to give bilayer phase transition temperatures.				
14. SUBJECT TERMS phosphocholines; stereoisomers; surfactants; vesicles		15. NUMBER IF PAGES 5		
		16. PRICE CODE		
17. SECURITY CLASSIFICATION OR REPORT UNCLASSIFIED	18. SECURITY CLASSIFICATION OF THIS PAGE UNCLASSIFIED	19. SECURITY CLASSIFICATION OF ABSTRACT UNCLASSIFIED	20. LIMITATION OF ABSTRACT UL	

Stereochemical Dependence of Vesicle Bilayer Properties

Final Progress Report

by

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October 14, 1996

U.S. Army Research Office
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Summary of Completed Project

A. Statement of the Problem Studied.

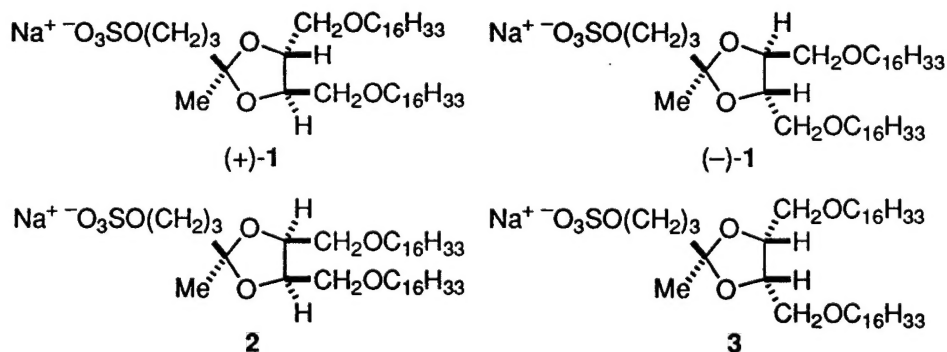
The project involved several studies of surfactants and their derived vesicles. The primary objective of the project was an elucidation of factors involved in the stabilization and destabilization of vesicles. Work was performed on three studies. The first involved the dependence of the properties of vesicles on the stereochemistry of their constituent surfactants. The second and third studies involved the synthesis of double-chain, double-head group surfactants and chain-substituted phosphocholines, respectively, and characterization of their derived vesicles.

B. Technical Information.

STUDY 1. Stereochemical Dependence of Vesicle Character on Surfactant Stereochemistry.

The properties of surfactant-derived bilayer vesicles (and bilayers in general) depend upon both their hydrocarbon bilayer interiors and bilayer-water interfaces. The involvement of the latter, wherein the surfactant head groups reside, suggests that differences among the head groups of stereoisomeric surfactants could have an effect on vesicular properties. If the full potential of vesicles as entrapment and release devices is to be realized, it is important to delineate the influence on their properties of such subtle features as the stereochemistry of their constituent surfactants.

We performed a study¹ of all of the stereoisomeric forms of surfactants **1-3**, which contain two hydrocarbon chains and a sulfate head group: enantiomers (+)-**1** and (-)-**1**, derived from D-(-)-tartaric acid and L-(+)-tartaric acid, respectively; racemate (\pm)-**1**, derived from D,L-(\pm)-tartaric acid; and diastereomers *meso* **2** and **3**, derived from *meso*-tartaric acid. Surfactant (\pm)-**1** was also obtained by the admixture of equal amounts of (+)-**1** and (-)-**1**. The following question was addressed: How are their stereochemical differences expressed in vesicular form?

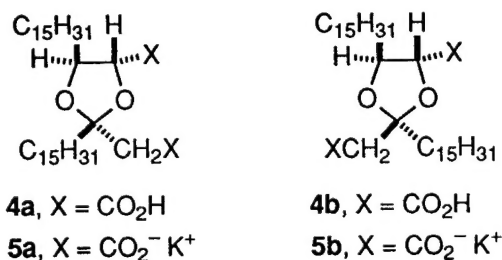


Vortexed vesicles of surfactants (+)-**1**, (-)-**1**, (\pm)-**1**, **2**, and **3** were studied by dynamic laser light scattering (DLS), to give vesicle sizes, and by differential scanning calorimetry (DSC), to give bilayer phase transition temperatures (T_c) and associated calorimetric enthalpies (ΔH_{cal}). Different DSC results were obtained for (+)-**1**[(-)-**1**], (\pm)-**1**, **2**, and **3**. In particular, a comparison of the results for (+)-**1**[(-)-**1**] and (\pm)-**1** indicated the operation of enantiomer discrimination. The same T_c value but slightly different ΔH_{cal} values were obtained in a comparison of (+)-**1** with (-)-**1**, and in that of (\pm)-**1** obtained from (\pm)-tartaric acid with (\pm)-**1** obtained by the admixture of equal amounts of (+)-**1** and (-)-**1**. The DLS results for (+)-**1** and (-)-**1** were very similar but not identical. The unexpected differences in the comparisons involving (+)-**1** with (-)-**1** and the samples of (\pm)-**1** obtained by the two different methods were likely due to the presence of (undetected) trace impurities in the surfactants, which were "pure" by several analytical methods. The results illustrate the difficulty that can be encountered in satisfying the criteria of the "absolute" method of stereochemistry in studies of chiral surfactants, even when due care has been taken. Overall, the results obtained with surfactants **1-3** indicate that the properties of their derived vesicles do indeed depend on stereochemistry.

STUDY 2. Double-Chain Surfactants with Two Head Groups that Form Vesicles.

Double-chain surfactants with two head groups comprise an active research area. The literature contains reports of examples with dianionic, dicationic, and dinonionic head groups. These surfactants exhibit interesting solution behavior, and, in particular, some dicationic systems display bactericidal activity greater than that of single-chain quaternary ammonium surfactants. Most double-chain surfactants with two head groups reported to date form micelles. Only a few form vesicles/bilayers. The aggregate morphologies of double-chain, double-head group surfactants are of interest because the established relationship between surfactant structure and morphology cannot always be routinely applied to them.

In this study² we synthesized diastereomeric diacids **4a** and **4b** and characterized the aggregates of derived surfactants **5a** and **5b**, which contain two carboxylate head groups and two alkyl chains connected by a ketal group. Diacids **4a** and **4b** were obtained and used as a 1:1 mixture. Surfactants **5a** and **5b** were obtained by dissolution of **4** into a pH 9.2 or 10.7 buffer. Sonication of **5** gave small unilamellar vesicles (SUVs), which were characterized by ¹H NMR spectroscopy, DLLS, DSC, and gel filtration chromatography.



By DLLS the SUVs were larger in the pH 9.2 buffer than in the 10.7 buffer, and by DSC the T_c value of vesicular **5** was higher in the former than in the latter buffer. On going from pH 9.2 to 10.7, the fraction of carboxylate groups among the carboxyl and carboxylate head groups increases, resulting in greater electrostatic repulsion among the head groups. As a consequence of greater head group repulsion, there is an increase in aggregate curvature and thus a smaller aggregate size at the higher pH. A decrease in the efficiency of alkyl chain packing also accompanies these changes, reflected by a decrease in T_c on going from pH 9.2 to 10.7.

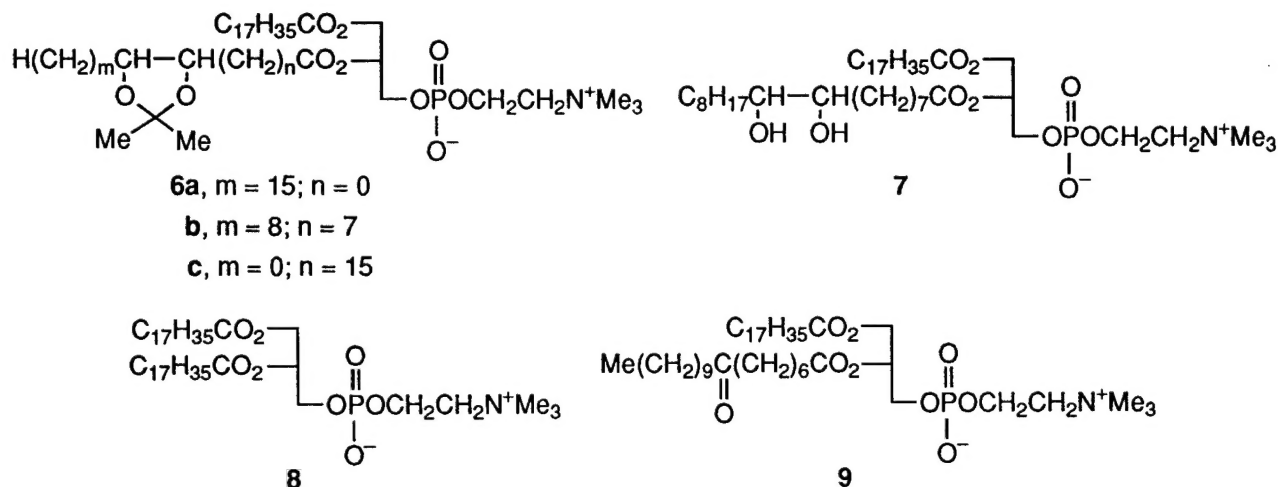
Surfactants **5** in principle represent cleavable surfactants since they contain a ketal group, which is stable under neutral and basic conditions, but labile under acidic conditions. The cleavable nature of **5** was not demonstrated because upon acidification of an aqueous solution of **5**, water-insoluble diacids **4** would precipitate before hydrolysis of the ketal group.

Overall, we synthesized and characterized in detail a rare example of a vesicle-forming surfactant bearing two alkyl chains and two head groups.

STUDY 3. Synthesis and Characterization of Chain-Substituted Phosphocholines.

The literature reports several studies of the properties of chain-substituted phosphocholines and related phospholipids. In particular, it has been generally found that (small) nonpolar substituents located at/near the beginning or end of one or both acyl chains of a saturated phosphocholine have only modest effects on its main T_c value, whereas mid-chain nonpolar substituents have substantial effects. In contrast, the effects of (polar) keto substituents on the T_c values of saturated phosphocholine bilayers are relatively insensitive to their positions along one or both acyl chains. The T_c values of phosphocholines with mid-chain keto groups are in fact comparable to that of the parent phosphocholine. The goal of this study was to determine if other polar substituents similarly affect the T_c values of phosphocholine bilayers and if they affect the sizes and population distributions of derived vesicles.

We performed a DSC and DLLS study³ of phosphocholines **6** and **7**. In **6**, a ketal group is located at the beginning (**6a**), middle (**6b**), or end (**6c**) of a C18 acyl group at carbon-2 of the glycerol backbone. In **7**, the middle of a C18 acyl group contains a *vic*-diol group. Uniformly, within **6a**, **6b**, and **7** the stereochemistry of the 2-acyl substituent is *threo*. Among themselves, ketal groups, which contain polar carbon-oxygen bonds, can participate in dipole-dipole interactions, whereas the more polar *vic*-diol groups can participate in (stronger) hydrogen bonding interactions. The DSC and DLLS results for **6** and **7** were compared to those for the parent phosphocholine **8**.



Phosphocholines **6** and **7** formed vesicles with sizes and population distributions similar to those of **8**. Phosphocholines **6a** and **6c** gave T_c and ΔH_{cal} values comparable to those of **8**, whereas **6b** gave much lower T_c and ΔH_{cal} values. The calorimetric behavior of **7** depended on the scan rate. In fast scans it gave T_c and ΔH_{cal} values about the same as, and greater than, respectively, those of **8**. In subsequent slow scans the same sample gave a single endotherm in the first scan and, at most, only a trace of an endotherm in the second scan. The loss of the endotherm is likely due to slight decomposition of **7**.

It is interesting to note that the mid-chain ketal groups do not stabilize the bilayer of **6b**, in contrast to the stabilization provided by the mid-chain *vic*-diol and keto groups of **7** and previously reported **9**, respectively. Within a bilayer, hydrogen bonding is possible among the *vic*-diol groups of **7**, as noted above, whereas only dipole-dipole interactions are possible among both the ketal groups of **6b** and the keto groups of **9**. Given the bilayer stabilization provided by the latter, the lack of stabilization by the former groups cannot be attributed to their inability to hydrogen bond among themselves. But the dipole-dipole interactions among the ketal groups are almost certainly weaker than those among the keto groups. It is therefore apparent that not all polar mid-chain substituents are effective in stabilizing a phosphocholine bilayer. Whether or not such a substituent provides stabilization depends upon its detailed nature.

To the extent that the T_c values of bilayers of closely related lipids reflect their relative permeabilities, this study provides useful information about the effect of phosphocholine structure on bilayer permeability. Also, if an increase in the permeability of **7**'s bilayers accompanies the thermally-induced disappearance of its phase transition, vesicular **7** could be used as an entrapment and release device.

References

- (1) Jaeger, D. A.; Brown, E. L. G. *Langmuir* **1996**, *12*, 1976.
- (2) Jaeger, D. A.; Kubicz-Loring, E.; Price, R. C.; Nakagawa, H. *Langmuir*, in press.
- (3) Jaeger, D. A.; Subotkowski, W.; Wang, J.; Kubicz-Loring, E.; Shinozaki, H. *Langmuir*, submitted for publication.

C. List of Publications.

1. Brown, E. *Cleavable Double-Chain, Double-Head Group Surfactants*, M.S. Thesis, University of Wyoming, December 1994.

The other publications that derived from the (partial) support provided by this grant correspond to the three publications listed above as references. They have been reported as those of the parent grant: DAAH04-94-G-0347, "Demilitarization of Mustard and Vesicles for Chemical Reagent Storage and Release".

D. Reportable Inventions.

None

E. List of All Participating Scientific Personnel.

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E. Brown was awarded an M.S. degree in December 1994.